

# SEQUENCE LISTING

<110> Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo

<120> Biologically-active conjugate

<130> US945

<160> 14

<210> 1

<211> 157

<212> PRT

<213> human

<400> 1

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      20             25             30
Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu
      35             40             45
Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe
      50             55             60
Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
      65             70             75             80
Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala
      85             90             95
Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys
      100            105            110
Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys
      115            120            125
Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe
      130            135            140
Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
      145            150            155

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<210> 2

<211> 157

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant protein of human tumor necrosis factor

<400> 2

Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Xaa Pro Val Ala His Val  
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Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg  
20 25 30  
Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu  
35 40 45  
Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe  
50 55 60  
Xaa Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile  
65 70 75 80  
Ser Arg Ile Ala Val Ser Tyr Gln Thr Xaa Val Asn Leu Leu Ser Ala  
85 90 95  
Ile Xaa Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Xaa  
100 105 110  
Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Xaa  
115 120 125  
Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe  
130 135 140  
Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu  
145 150 155

<210> 3

<211> 157

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant protein of human tumor necrosis factor

<400> 3

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Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Met Pro Val Ala His Val
 1             5             10             15
Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg
      20             25             30
Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu
      35             40             45
Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe
      50             55             60
Ser Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
      65             70             75             80
Ser Arg Ile Ala Val Ser Tyr Gln Thr Pro Val Asn Leu Leu Ser Ala
      85             90             95
Ile Arg Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Asn
      100             105             110
Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Pro
      115             120             125
Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe
      130             135             140
Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
      145             150             155
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<210> 4

<211> 92

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

<400> 4

tctactccca ggtcctcttc nnsggccaag getgccctc caccatgtg ctctcacc 60

acaccatcag ccgcacgcc gtctcctacc ag

<210> 5

<211> 90

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

<400> 5

ggcctcagcc ccctctgggg tctccctctg gcaggggcts nngatggcag agaggagggt 60  
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<210> 6

<211> 110

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

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<210> 7

<211> 95

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

<400> 7

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<210> 8

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

<400> 8

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<210> 9

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as primer with NNS sequence

<400> 9

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<210> 10

<211> 58

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as linker to insert into an expression vector a cDNA  
coding  
a variant protein of human tumor necrosis factor

<400> 10

gtttaacttt aagaaggaga tatacatatg gtcagatcat cttctcgaac cccgagtg

<210> 11

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used as linker to insert into an expression vector a cDNA coding

a variant pritein of human tumor necrosis factor

<400> 11

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<210> 12

<211> 471

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA coding a variant protein of human tumor necrosis factor

<400> 12

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Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Met Pro Val Ala His Val

1

5

10

15

gta gca aac cct caa gct gag ggg cag ctc cag tgg ctg aac cgc cgg 96

Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg

20

25

30

gcc aat gcc ctc ctg gcc aat ggc gtg gag ctg aga gat aac cag ctg 144

Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu

35

40

45

gtg gtg cca tca gag ggc ctg tac ctc atc tac tcc cag gtc ctc ttc 192

Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe

50

55

60

tgc ggc caa ggc tgc ccc tcc acc cat gtg ctc ctc acc cac acc atc 240

Ser Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile  
 65 70 75 80  
 agc cgc atc gcc gtc tcc tac cag acc ccc gtc aac ctc ctc tct gcc 288  
 Ser Arg Ile Ala Val Ser Tyr Gln Thr Pro Val Asn Leu Leu Ser Ala  
 85 90 95  
 atc cgc agc ccc tgc cag agg gag acc cca gag ggg gct gag gcc aac 336  
 Ile Arg Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Asn  
 100 105 110  
 ccc tgg tat gag ccc atc tat ctg gga ggg gtc ttc cag ctg gag ccg 384  
 Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Pro  
 115 120 125  
 ggt gac cga ctc agc gct gag atc aat cgg ccc gac tat ctc gac ttt 432  
 Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe  
 130 135 140  
 gcc gag tct ggg cag gtc tac ttt ggg atc att gcc ctg  
 Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu  
 145 150 155

<210> 13

<211> 471

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA coding a variant protein of human tumor necrosis factor

<400> 14

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 Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg  
 20 25 30  
 gcc aat gcc ctc ctg gcc aat ggc gtg gag ctg aga gat aac cag ctg 144  
 Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu

35	40	45	
gtg gtg cca tca gag ggc ctg tac ctc atc tac tcc cag gtc ctc ttc 192			
Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe			
50	55	60	
tcg ggc caa ggc tgc ccc tcc acc cat gtg ctc ctc acc cac acc atc 240			
Ser Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile			
65	70	75	80
agc cgc atc gcc gtc tcc tac cag acc cgc gtc aac ctc ctc tct gcc 288			
Ser Arg Ile Ala Val Ser Tyr Gln Thr Arg Val Asn Leu Leu Ser Ala			
	85	90	95
atc gcc agc ccc tgc cag agg gag acc cca gag ggg gct gag gcc ctc 336			
Ile Ala Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Leu			
100	105	110	
ccc tgg tat gag ccc atc tat ctg gga ggg gtc ttc cag ctg gag acc 384			
Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Thr			
115	120	125	
ggt gac cga ctc agc gct gag atc aat cgg ccc gac tat ctc gac ttt 432			
Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe			
130	135	140	
gcc gag tct ggg cag gtc tac ttt ggg atc att gcc ctg			
Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu			
145	150	155	

<210> 14

<211> 157

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant protein of human tumor necrosis factor

<400> 3

Val	Arg	Ser	Ser	Ser	Arg	Thr	Pro	Ser	Asp	Ala	Pro	Val	Ala	His	Val
1				5				10				15			
Val	Ala	Asn	Pro	Gln	Ala	Glu	Gly	Gln	Leu	Gln	Trp	Leu	Asn	Arg	Arg
			20					25							30



Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu  
 35 40 45  
 Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe  
 50 55 60  
 Ser Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile  
 65 70 75 80  
 Ser Arg Ile Ala Val Ser Tyr Gln Thr Arg Val Asn Leu Leu Ser Ala  
 85 90 95  
 Ile Ala Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Leu  
 100 105 110  
 Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Thr  
 115 120 125  
 Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe  
 130 135 140  
 Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu  
 145 150 155